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Molecular and cellular mechanisms of liver fibrosis and its regression

Tatiana Kisseleva[1] ✉ *and David Brenner*[2]

Abstract | Chronic liver injury leads to liver inflammation and fibrosis, through which activated myofibroblasts in the liver secrete extracellular matrix proteins that generate the fibrous scar. The primary source of these myofibroblasts are the resident hepatic stellate cells. Clinical and experimental liver fibrosis regresses when the causative agent is removed, which is associated with the elimination of these activated myofibroblasts and resorption of the fibrous scar. Understanding the mechanisms of liver fibrosis regression could identify new therapeutic targets to treat liver fibrosis. This Review summarizes studies of the molecular mechanisms underlying the reversibility of liver fibrosis, including apoptosis and the inactivation of hepatic stellate cells, the crosstalk between the liver and the systems that orchestrate the recruitment of bone marrow-derived macrophages (and other inflammatory cells) driving fibrosis resolution, and the interactions between various cell types that lead to the intracellular signalling that induces fibrosis or its regression. We also discuss strategies to target hepatic myofibroblasts (for example, via apoptosis or inactivation) and the myeloid cells that degrade the matrix (for example, via their recruitment to fibrotic liver) to facilitate fibrosis resolution and liver regeneration.

Chronic liver inflammation leads to fibrosis, cirrhosis and the development of hepatocellular carcinoma (HCC)[1]. Hepatic fibrosis is the formation of a fibrous scar that results from the accumulation of extracellular matrix (ECM) proteins, mostly crosslinked collagens type I and type III, that replace damaged normal tissue[1]. Hepatic fibrosis results from two general types of chronic liver injuries: hepatotoxic injury and cholestatic injury. Hepatotoxic injury is caused by the chronic injury of hepatocytes by, for example, hepatitis B (HBV) and/or hepatitis C (HCV) infections, alcohol, or metabolic syndrome inducing non-alcoholic steatohepatitis (NASH)[2]. Cholestatic injury is caused by obstruction to bile flow, such as in primary (and secondary) biliary cholangitis (PBC), primary sclerosing cholangitis (PSC) and biliary atresia[2].

Studies of patients with liver fibrosis and cirrhosis of different aetiologies along with experimental models of liver fibrosis in rodents have revealed common key molecular mechanisms leading to liver fibrosis. These include chronic hepatocellular injury, damage to the epithelial or endothelial barrier, release of inflammatory cytokines, recruitment of bone marrow-derived inflammatory cells, production of TGFβ by macrophages, activation of collagen type I (COL1A1)-secreting hepatic myofibroblasts, excessive production of ECM (mostly collagen type I) and the formation of a fibrous scar[2].

One of the most important concepts in clinical and experimental liver fibrosis is regression. Many clinical studies have demonstrated that hepatic fibrosis can be reversible in patients (for example, in those with HBV or HCV infection, biliary obstruction or alcohol abuse)[3] and in experimental rodent models (for example, alcohol feeding, CCl_4 or bile duct ligation (BDL))[3–5]. Upon removal of the aetiological source of the chronic injury, regression of liver fibrosis is associated with a decreased production of pro-inflammatory or profibrogenic cytokines, increased collagenolytic activity, disappearance of hepatic myofibroblasts, suppression of ECM production and dissolution of the fibrous scar[2,6]. For example, a sustained viral response to HCV treatment induces long-term regression of fibrosis, while patients receiving no treatment or with treatment failure had progressive increases in fibrosis markers[7]. This Review summarizes the molecular mechanisms underlying the reversibility of liver fibrosis, including apoptosis and inactivation of hepatic stellate cells (HSCs), the crosstalk between the liver and the recruitment of bone marrow-derived macrophages (and other inflammatory cells) that drive fibrosis regression, and the interactions between cell types that lead to the intracellular signalling that induces fibrosis or its regression. The strategies to target hepatic myofibroblasts and the myeloid cells that degrade the matrix to facilitate fibrosis resolution and liver repair are also discussed.

[1]*Department of Surgery, University of California, San Diego, La Jolla, CA, USA.*

[2]*Department of Medicine, University of California, San Diego, La Jolla, CA, USA.*

✉*e-mail: tkisseleva@ucsd.edu*

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Key points

- Most chronic liver diseases, such as hepatitis C virus infection or non-alcoholic hepatic steatohepatitis, can progress to liver fibrosis with the formation of a fibrous scar.
- Experimental and clinical liver fibrosis regresses with the removal of the aetiological agent or with new therapeutic interventions.
- Chronic liver injury leads to activation of hepatic stellate cells, the major source of the fibrous scar in liver fibrosis.
- Hepatic stellate cells have four known phenotypes — quiescent, activated, inactivated and senescent — each of which has a critical role in liver fibrosis and its regression.
- During regression of liver fibrosis, activated hepatic stellate cells can undergo apoptosis or revert to an inactivated phenotype; the inactivated cells have a phenotype that is similar to but distinct from quiescent hepatic stellate cells.
- Macrophages can promote fibrogenesis by the secretion of TGFβ and other agonists, but they also support the regression of fibrosis through the secretion of collagenases that resorb the fibrous scar.

Cell types in liver fibrosis

Myofibroblasts

Myofibroblasts, which are not present in the healthy liver[2,8], are activated in response to liver injury. Myofibroblasts are the primary source of ECM in fibrotic liver[8,9] and therefore represent a primary target for anti-fibrotic therapy. Myofibroblasts are characterized by a spindle or stellate shape and the expression of abundant intracellular proteins (vimentin, α-smooth muscle actin (αSMA) and non-muscle myosin). Ultrastructurally, myofibroblasts are identified by a rough endoplasmic reticulum and a Golgi apparatus producing collagen. The origin of myofibroblasts has been intensively studied, and several sources of hepatic myofibroblasts have been identified[10–13], including liver-resident cells, HSCs and portal fibroblasts and bone marrow-derived cells such as fibrocytes and mesenchymal stem cells (MSCs)[2,8,13] (BOX 1). Experimental models of liver fibrosis have provided no evidence for the contribution of liver parenchymal cells to the myofibroblast population[14,15].

The composition of myofibroblasts in fibrotic liver. The origin of myofibroblasts in clinical liver disease is difficult to identify. Electron microscopy and immunohistochemistry have provided evidence that both portal fibroblasts and HSCs can transform into myofibroblasts[9,16,17]. Cell fate mapping and deep phenotyping have demonstrated that experimental models of liver fibrosis, activated HSCs and activated portal fibroblasts comprise >90% of the collagen-producing cells, suggesting that these cells are the major sources of myofibroblasts[18]. However, the composition of myofibroblasts can vary substantially, depending on the aetiology of hepatic fibrosis. HSCs are primarily activated in response to toxic liver injury, which mostly affects the centrilobular and perisinusoidal regions in the liver (FIG. 1), whereas both activated portal fibroblasts and HSCs contribute to cholestatic liver fibrosis that results mostly from periportal injury[18,19]. Specifically, activated portal fibroblasts comprise 70% of myofibroblasts at the onset of experimental cholestatic injury (5 days after BDL), whereas HSCs are increasingly activated with the progression of cholestatic injury (17 days after BDL), eventually constituting the largest myofibroblast population (20 days after BDL)[18]. In addition, fibrocytes (discussed later) comprise a small population of bone marrow-derived myofibroblasts (<4%) in livers of mice with either toxic or cholestatic fibrosis[18,20] (BOX 1).

Alternative sources of myofibroblasts in fibrotic liver. Several other sources of hepatic myofibroblasts have been suggested. The epithelial-to-mesenchymal transition (EMT), a process in which mature epithelial cells transition into fully differentiated mesenchymal cells (fibroblasts or myofibroblasts), was implicated in the pathogenesis of liver fibrosis[21]. Although the EMT has an important role during embryonic development and tumorigenesis, cell fate mapping experiments in mice revealed no contribution of EMT to BDL-induced or CCl_4-induced liver fibrosis[14,15,22]. Bone marrow-derived mesenchymal progenitors (or MSCs[23]), defined as self-renewable multipotent progenitor cells[24–26] with the capacity to differentiate into lineage-specific cells that form bone, cartilage, fat, tendon and muscle tissue[24,27], were reported to populate fibrotic lung[28], kidney, and liver[23] and to give rise to tissue myofibroblasts[8,23,29]. Although the potential contribution of circulating MSCs to the population of fibrogenic myofibroblasts has not been ruled out, heterologous transplantation of human MSCs from healthy donors was reported to improve liver fibrosis via the suppression of the inflammatory cytokines[30–32]. A few clinical trials using autologous MSCs have been performed in patients with cirrhosis caused by HCV, HBV or alcoholic liver disease (ALD), with a modest improvement of clinical parameters

Box 1 | Markers for myofibroblasts in the liver

Liver-resident activated hepatic stellate cells and activated portal fibroblasts are believed to be the major source of fibrogenic myofibroblasts in the fibrotic liver. Bone marrow-derived fibrocytes also contribute to a small population of collagen type I-producing cells. Bone marrow-derived mesenchymal stem cells might potentially differentiate into myofibroblasts, but their contribution to hepatic fibrosis remains controversial. The epithelial-to-mesenchymal transition (EMT) was also suggested to contribute to fibrogenic myofibroblasts; however, fate cell mapping experiments did not support this concept. Specific markers for hepatic myofibroblasts from different sources that distinguish activated hepatic stellate cells from activated portal fibroblasts, fibrocytes and EMT-associated myofibroblasts are listed.

EMT associated[14,15,22,278–280]

- $CD45^-$, $Albumin^+/CK19^+$, $FSP1^+$, $COL1A1^+$, αSMA^+

Liver resident[58,281]

Quiescent to activated HSCs

- $Desmin^+$, $CD146^+$, $CD105^+$, $GFAP^+$, $LRAT^+$, $Synemin^+$, $Synaptophysin^+$, $p75\ (NGFR)^+$, $PDGFR\beta1^+$, $PPAR\gamma^+$, $Adipor1^+$, $ADFP1^+$, $CD36^+$, $Cytoglobin^+$, $SPP1^{+,a}$, $LOX^{+,a}$, $LOXL2^{+,a}$, $NR1D2^{+,a}$, $IL\text{-}17RA^{+,a}$, $COL1A1^{+,a}$, $\alpha SMA^{+,a}$

Activated portal fibroblasts[18,20,38,80,282–284]

- $THY1^+$, $Elastin^+$, $CD105^+$, $CD45^-$, $CD34^-$, $Desmin^-$, $GFAP^-$, $Cofilin^+$, $Fibulin2^+$, $Gremlin^+$, $NTPD2^+$, $Smoothelin^+$, $Calcitonin\ \alpha^+$, $Mesothelin^+$, $uroplakin\ 1\beta^+$, $basonuclin\ 1^+$, $Asporin^+$, $Vitrin^+$, $IL\text{-}18R1^+$, $COL1A1^{+,a}$, $COL15A1^{+,a}$, $\alpha SMA^{+,a}$

Bone marrow derived

Fibrocytes[81,88,90,285–287]

- $COL1A1^+$, $CD45^+$, $CD34^+$, $CD11b^+$, $GR1^+$ or $LY6G^+$, $25F9^+$, $S100A8/9^+$, $MHCII^+$, $CD80^+$, $CD86^+$, $CCR2^+$, $CCR7^+$, $CXCR4^+$, $ICAMI^+$, αSMA^+

Mesenchymal stem cells[23,288–290]

- LIN^-, $CD45^-$, $CD31^-$, $CD34^-$, $CD133^-$, $SCA1^+$, $CD146^+$, $COL1A1^{\pm}$, $\alpha SMA^{\pm}$

[a]Indicates the expression of fibrogenic genes. Data derived from REFS[14,15,22,23,272,288–290]

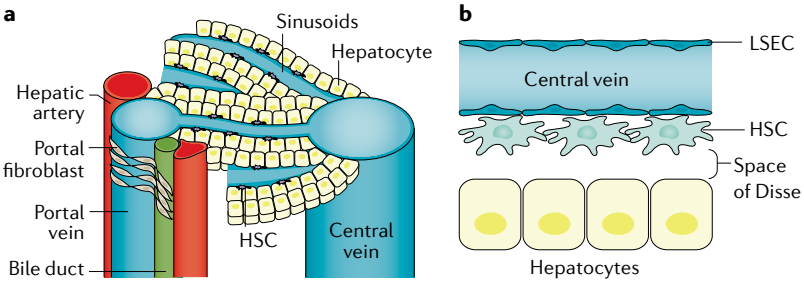

Fig. 1 | **Portal fibroblasts and HSCs are located in different areas of the hepatic lobule.** **a** | The structure of the hepatic lobule. The central vein, hepatic artery, portal vein and bile duct are shown. Under physiological conditions, portal fibroblasts are located around portal triads. **b** | Quiescent hepatic stellate cells (HSCs) are located in the space of Disse, a designated area between sinusoidal endothelial cells (LSEC) and hepatocytes clusters.

(levels of albumin or creatinine) without severe adverse effects, suggesting a potential efficacy of MSC therapy for liver disease[33].

Hepatic stellate cells

Under physiological conditions, HSCs reside in the space of Disse, contain retinoid lipid droplets and exhibit a quiescent phenotype. Quiescent HSCs function as pericytes and as the major storage site of vitamin A[29,34,35], which is essential for the maintenance of the quiescent HSC phenotype[36,37]. In response to injury, quiescent HSCs downregulate the expression of vitamin A, GFAP, and PPARγ and become activated HSCs (FIG. 2), which are equivalent to collagen type I-producing myofibroblasts[13]. Following stimulation with fibrogenic mediators, HSCs upregulate the expression of α-smooth muscle actin and other myofibroblast intracellular microfilaments (FIG. 2). Activated HSCs migrate to the site of injury and secrete ECM to produce a fibrous scar[18,38] (FIG. 3). Several cytokines can directly stimulate collagen type I transcription in activated HSCs. TGFβ is the most potent profibrogenic cytokine and drives HSC activation in a SMAD2-dependent or SMAD3-dependent manner[39]. *Col1a1*, *Col1a2*, *Activin* and *Pai1* genes were identified as TGFβ targets in activated HSCs in mice[40]. Leptin, IL-6 and IL-17 can also induce COL1A1 transcription via JAK–STAT3 signalling[41]. In addition, connective tissue growth factor (CTGF) and IL-13 facilitate COL1A1 expression in activated HSCs via a TGFβ1-independent pathway[42]. The liver is capable of remarkable regeneration following injury, and liver cells seem capable of plasticity under different stresses (discussed later). HSCs transplanted into the injured rat liver have been reported to transform into progenitor cells or hepatocytes or to a hepatocyte-like cell[43]. Furthermore, gene transfer of hepatocyte transcription factors can convert a small population of activated HSCs into hepatocyte-like cells in the fibrotic liver of mice[4,44]. However, cell fate mapping by genetic labelling has clearly demonstrated that endogenous HSCs are not converted to hepatic parenchymal cells following liver injury[4,44].

Hepatocytes

In response to injury, hepatocytes change their gene expression and secretion profile. Some of these newly expressed factors are fibrogenic, including Notch[45], osteopontin, TGFβ, NADPH oxidase 4, TAZ and Indian Hedgehog[46–48]. Furthermore, injured hepatocytes but not normal hepatocytes secrete exosomes that can contain micro RNAs that activate HSCs[49]. However, as many studies have demonstrated that inflammation is an absolute requirement for fibrosis, factors from injured hepatocytes alone are not sufficient to directly activate HSCs and lead to fibrosis.

Inflammatory cells

Inflammation has a critical role in the pathogenesis of liver fibrosis. Neutrophils are often recruited to the damaged liver and act as first responders to clear apoptotic hepatocytes[38,50]. These cells serve as a source of cytokines and also entrap and kill bacteria by ejecting nuclear or mitochondrial DNA nets, along with the release of antimicrobial enzymes[51]. Neutrophils release cell-free DNA, which itself has a strong pro-inflammatory effect[38,52]. The ablation of neutrophils[53] or the deletion of neutrophil chemoattractants (IL-8, IL-18 and IL-17 (which regulates IL-8), CCL3, CCL4 and CXCL2) attenuated the development of liver fibrosis in mice[54–56].

The activation of TGFβ-producing macrophages has a key role in the pathogenesis of liver fibrosis. It is believed that bone marrow-derived macrophages and not liver-resident macrophages (Kupffer cells) are the major source of TGFβ[57]. Although still unresolved, Kupffer cells have been reported to produce TGFβ but also to possess phagocytic and anti-inflammatory functions[58,59]. Genetic deletion of myeloid TGFβ[60] or pharmacological blocking of TGFβ[61] ameliorated liver fibrosis in mice, whereas genetic overexpression of TGFβ spontaneously induced fibrosis in targeted tissues and organs[62], including the liver, suggesting that TGFβ is a key mediator of fibrosis. However, long-term depletion of TGFβ is associated with severe adverse effects such as poor wound healing and increased carcinogenesis in mice[63]. Furthermore, genetic deletion of IL-6, TNF or IL-1β attenuates the development of liver fibrosis as these cytokines can be synergistic with TGFβ[64,65]. Lipopolysaccharide (LPS), IL-1β and TNF can potentiate signalling of TGFβ by downregulating BAMBI, an inhibitory TGFβ pseudo-receptor[58,66]. TGFβ and IL-6 are critical regulators of naive T cell differentiation towards T helper 17 (T_H17) cells[65,67]. Mouse T_H17 cells produce highly profibrogenic IL-17A and anti-inflammatory IL-22. Genetic ablation of IL-17 signalling (using *Il17ra*$^{-/-}$ or *Il17a*$^{-/-}$ mice) strongly attenuated the development of liver fibrosis[41], whereas IL-22 acts as a survival factor for hepatocytes[68], suggesting that the activation of T cells might also reduce liver injury by releasing the hepatoprotective cytokine IL-22 (REF.[69]).

Macrophages play dual roles in liver fibrosis progression and its resolution. During the progression of fibrosis, injury-induced inflammation triggers the recruitment of macrophages to the liver, where they produce cytokines and chemokines to induce the activation of HSCs[29]. CCL2, which is secreted by Kupffer cells and HSCs, facilitates the recruitment of immature monocyte-derived LY6C^{hi} macrophages to the liver[70]. The deletion of macrophages in CD11b-DTR transgenic mice led to reduced fibrosis and fewer myofibroblasts in

CCl_4-induced injury[71]. However, during the regression of liver fibrosis, macrophages have a LY6C^{low} phenotype, stop the production of fibrogenic and inflammatory factors, and instead secrete matrix metalloproteinases (MMPs) such as MMP9 and MMP12 (REF.[72]). These enzymes are secreted by many cell types, including macrophages, and require post-translational modification for their activation[71]. Although a reduction in myofibroblasts decreases the production of ECM, increased collagenolytic activity is another primary mechanism of fibrosis resolution. The conversion of macrophages and the production of MMPs help to degrade and phagocytose existing ECM during regression of liver fibrosis[71]. Accordingly, the depletion of macrophages during recovery from CCl_4-induced liver fibrosis led to a failure of ECM degradation[71]. Additionally, myofibroblasts are the major source of TIMP production. A decline in myofibroblasts leads to reduced TIMP levels and contributes to increased MMP activities and degradation of the existing ECM[73].

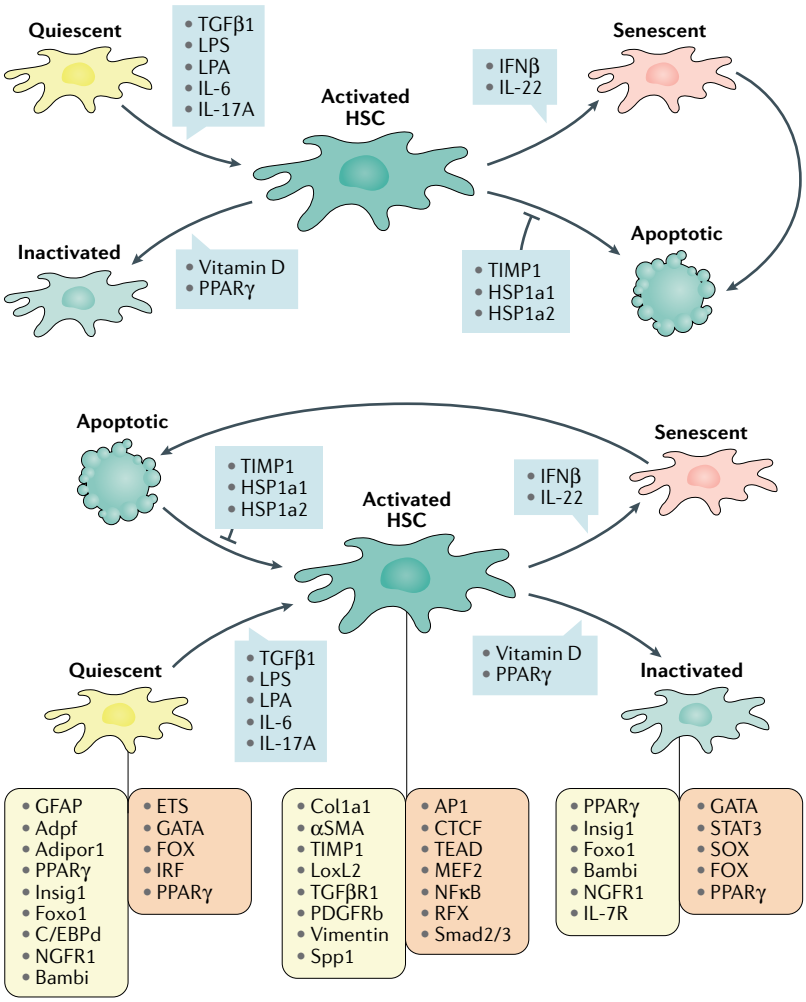

Fig. 2 | **Phenotypic changes in HSCs during development and regression of liver fibrosis.** Under physiological conditions, hepatic stellate cells (HSCs) exhibit a quiescent phenotype but, in response to fibrogenic stimuli, such as TGFβ1, HSCs undergo activation into activated HSCs (activated myofibroblasts). Upon cessation of fibrogenic stimuli, about half of the activated HSCs undergo apoptosis. IFNβ1 and IL-22 promote the senescence of activated HSCs. The overexpression of TIMP1 or HSP1a1/2 prevents activated HSC apoptosis. Those activated HSCs that escape apoptosis undergo inactivation and obtain a quiescent-like phenotype. Increased levels of vitamin D and re-expression of PPARγ in activated HSCs facilitate HSC inactivation. Genes (yellow boxes) and regulatory transcription factors (red boxes) that characterize each HSC phenotype (based on experimental models of toxic liver injury) are shown. For example, quiescent HSCs express neural (Lrat and NGFR1) and lipogenic (PPARγ, Adipor1 and others) genes. Chronic injury activates HSCs into collagen type I-producing activated HSCs or activated myofibroblasts. Phenotypic changes in HSCs are regulated on an epigenetic level. For example, specific modifications in the chromatin structure affect DNA accessibility of the regulatory transcription factors, causing transcriptional activation or repression of their target genes.

Liver sinusoidal endothelial cells

In the healthy liver, liver sinusoidal endothelial cells (LSECs) are fully differentiated, contain fenestrae and are highly endocytic[74]. Normal LSECs promote the quiescence of HSCs via paracrine factors, including nitric oxide[75,76]. In fibrotic livers in humans and in rats, LSECs lose their fenestrae and undergo capillarization[74,77]. Unlike normal LSECs, these capillarized LSECs do not prevent or revert HSC activation[75,78]. One potential mechanism for capillarization and HSC activation is that liver injury recruits LSEC progenitors from the bone marrow that engraft but fail to differentiate, enabling the activation of quiescent HSCs[78]. The LSEC response to liver injury might determine whether the result is liver regeneration or fibrosis. In particular, the CXCR7–ID1 pathway in LSECs in response to injury promotes liver regeneration, whereas the FGFR1–CXCR4 pathway promotes HSC activation and fibrosis[79].

Portal fibroblasts

Under physiological conditions, portal fibroblasts comprise a small population of cells that surround the portal venules, providing a supporting function to maintain the physical integrity of the portal tract (FIG. 1). First described as mesenchymal cells that are distinct from sinusoidal endothelial cells, activated portal fibroblasts have been referred to as periductular fibroblasts and portal or periportal mesenchymal cells[20] and implicated in the pathogenesis of cholestatic liver injury in experimental models and in humans[80]. Activated portal fibroblasts have several defining markers (FIG. 2, BOX 1). Similar to activated HSCs, TGFβ is critical for the activation of collagen type I production by activated portal fibroblasts. The interaction between mesothelin (MSLN), mucin 16 (MUC16) and THY1 surface receptors was shown to regulate TGFB1–TGFBRI–SMAD2/3-induced fibrogenic responses in activated mouse portal fibroblasts and proliferation via MSLN–AKT–FGFR1-dependent signalling[38]. This finding suggests that MSLN (a glycosyl phosphatidyl inositol-anchored membrane protein) and its ligand MUC16 (human analogue ligand CA125) might play an important part in activated portal fibroblast biology. Unlike activated HSCs, activated portal fibroblasts lack responses to PDGFβ and NGF[18]. In response to cholestasis, taurocholic acid has a direct stimulatory effect on COL1A1 expression in activated portal fibroblasts[18]. Furthermore, IL-25-stimulated activated portal fibroblasts secrete IL-13, which in turn facilitates the release of CTGF and subsequent HSC activation[18,42]. This understanding might explain why cholestasis (BDL)-activated HSCs exhibit more

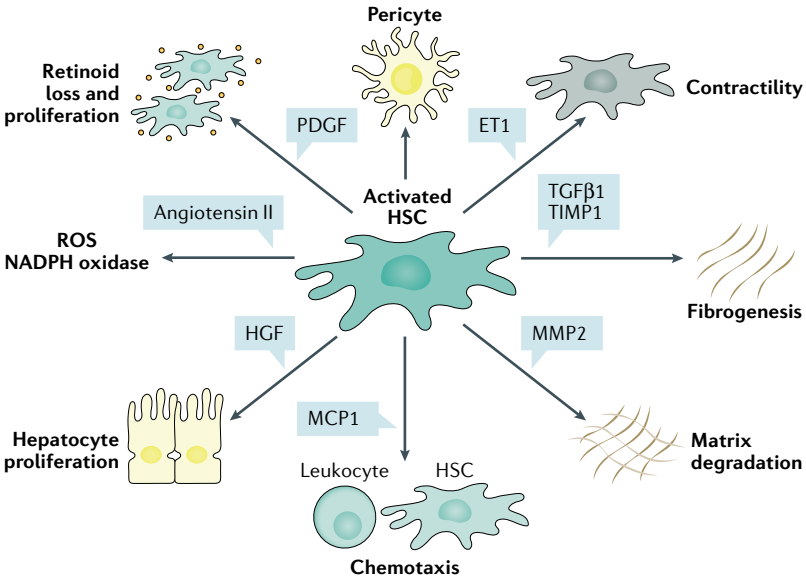

Fig. 3 | **Characteristics of activated HSCs.** In response to profibrogenic stimuli, activated hepatic stellate cells (HSCs) lose lipid droplets and obtain a myofibroblastic phenotype, acquire contractile capability and differentiate into collagen type I-producing cells expressing fibrogenic markers (α-smooth muscle actin, c-mub and myocyte enhancer factor 2). NADPH oxidase is a key downstream signalling mediator of angiotensin II in activated HSCs. Activated HSCs induce lymphocyte recruitment and control matrix degradation. However, the function of activated HSCs is not limited to collagen production. Studies have demonstrated that HSCs might be important for immune reactions in the injured liver. Activated HSCs secrete inflammatory cytokines (such as TGFβ, MCP1, RANTES, IL-10 and IL-8) and upregulate adhesion molecules (VCAM1 and ICAM1) that cause lymphocyte recruitment and activation. ROS, reactive oxygen species.

similarities in gene expression with activated portal fibroblasts than with HSCs activated in response to toxic injury (CCl_4)[18], suggesting that targeting activated portal fibroblasts might provide new directions for antifibrotic therapies.

Fibrocytes

Fibrocytes originate from haematopoietic stem cells and are defined as spindle-shaped $CD45^+$ and collagen type I-expressing leukocytes that mediate tissue repair and are capable of antigen presentation to naive T cells[81]. Due to their ability to differentiate into myofibroblasts, fibrocytes are implicated in the fibrogenesis of skin, lungs, kidneys and liver[82–84]. In addition to collagen type I, fibronectin and vimentin, fibrocytes express and secrete growth factors (TGFβ and MCP1) that promote deposition of ECM[85]. Upon injury or stress, fibrocytes proliferate and migrate to the injured organ[81,86]. The number of recruited fibrocytes has been reported to vary from 25%[87] (lung fibrosis) to 3–5% (liver fibrosis; from BDL and CCl_4 models)[10,88,89] of the collagen-expressing cells, suggesting that the magnitude of fibrocyte differentiation into myofibroblasts depends on the organ and the type of injury[90].

Pathogenesis of liver fibrosis

The development of liver fibrosis is characterized by excessive deposition of ECM. Despite the similarities in the end-stage pathology of a scarred liver with distorted architecture, the development of hepatotoxic and cholestatic liver fibrosis depends on the aetiology of the underlying liver disease.

Toxin-induced liver injury

The leading causes of hepatotoxic-induced liver fibrosis in the developed world are chronic viral infections, alcohol and metabolic syndrome. Chronic HBV and HCV infections were the main cause of liver fibrosis during the past two decades[91]. With the development of new, highly active antiviral treatments, the incidence of HBV-related and HCV-related liver cirrhosis[92] has declined in the USA, whereas NASH-related[93] and alcoholic steatohepatitis-associated liver cirrhosis and HCC[94] are rising. Although the global burden of cirrhosis is high, there is insufficient information to determine aetiologies[95]. HBV-associated and HCV-associated liver fibrosis have been reviewed elsewhere[96,97] and will not be further discussed here.

Approximately 60–80 million Americans will develop non-alcoholic fatty liver disease and 20% of these patients will progress to NASH[98], which is associated with metabolic syndrome, obesity and insulin resistance[4–6]. The pathology of NASH is characterized by steatohepatitis, ballooning degeneration of hepatocytes, lobular inflammation and peri-sinusoidal fibrosis or cirrhosis[7] (that distinguish non-alcoholic fatty liver disease from NASH by liver biopsy[99]).

The pathogenesis of NASH is often explained by a 'two-hit' theory[100]. The first hit is caused by dietary-induced obesity, excessive accumulation of triglycerides, fatty acids or cholesterol in steatotic hepatocytes (associated with the activation of transcription factors PPARγ, SERBP1, SERBP2 and expression of caspase 2)[101], and the dysregulation of adiponectin, leptin or resistin release[102], which results in increased insulin resistance and inflammation. The driver of the second hit is unclear, but it is associated with the production of reactive oxygen species (ROS), gut-derived LPS and further release of pro-inflammatory (TNF, IL-6, IL-1β, IL-17 and leptin) and profibrogenic (TGFβ) cytokines, which lead to HSC activation[100,103,104]. In addition, dietary supplements, such as fructose, directly increase intestinal permeability, resulting in bacterial products (such as LPS) being released into the portal circulation and the activation of Toll-like receptor (TLR) signalling, inflammation and fibrosis[105–107].

Patients with NASH and experimental NASH models have alterations in the composition of the gut microbiota, including reductions and increases in the abundance of Bacteroidetes and Firmicutes, respectively[108]. Manipulation of the gut microbiota with antibiotics or prebiotics, either broadly[58] or specifically[109], decreases experimental liver fibrosis, leading to the concept of a fibrosis-associated intestinal microbiota. The composition of the intestinal microbiota in experimental NASH increases the production of IL-17 (REF.[110]), suggesting that there is a correlation between NASH-induced dysbiosis, immune response and liver fibrosis. Thus, IL-17 is a common mediator in models of experimental liver fibrosis. Reinforcing an immune component to NASH, NASH development was linked to the accumulation of liver-resident IgA-producing (IgA^+) cells that directly

suppress antifibrogenic, cytotoxic $CD8^+$ T cells via the expression of PDL1 (REF.[111]). In support of this finding, the ablation of $CD8^+$ T cells exacerbated NASH-induced liver fibrosis, whereas genetic or pharmacological suppression of IgA^+ cells attenuated NASH-induced liver fibrosis in mice[111].

ALD is a major cause of cirrhosis and liver failure. ALD progresses from steatosis to steatohepatitis, fibrosis and HCC[56]. Alcohol induces liver injury directly (via acetaldehyde, a toxic ethanol metabolite produced by hepatocytes) and indirectly (via cytochrome P4502E1, an alcohol-metabolizing enzyme)[112]. Alcohol activates SREBP1-dependent or SREBP2-dependent synthesis of fatty acids and cholesterol[56], leading to the accumulation of hepatic fat droplets, centrilobular ballooning and the formation of Mallory–Denk bodies in alcohol-damaged hepatocytes[113,114]. Hepatotoxic injury by alcohol is associated with the upregulation of IL-8, IL-17 and CXCL1 (REFS[115–117]), neutrophilic infiltration, recruitment of bone marrow-derived or liver-resident macrophages to the alcohol-injured liver, the secretion of TGFβ, and the activation of myofibroblasts that secrete excessive ECM proteins leading to liver fibrosis[2,58,113,114,118]. In contrast to the effects of a high-fat diet in inducing NASH, formation of the ethanol metabolite acetaldehyde can directly stimulate collagen type I production by activated HSCs[119]. T cells and B cells are also recruited to the alcohol-injured liver (D.B. and T.K., unpublished observations); however, their role in the pathogenesis of ALD-induced liver fibrosis remains unresolved. Increased intestinal permeability and intestinal microbial dysbiosis are also early changes in experimental ALD and drive liver injury[120].

Cholestatic liver injury

Cholestasis is defined as a decrease in bile flow due to impaired secretion by hepatocytes or due to the obstruction of bile flow. The mechanisms that result in chronic cholestasis progressing to liver fibrosis are under investigation. Cholestatic liver fibrosis is caused by genetic defects (such as Alagille syndrome), mechanical injury of the bile ducts (such as biliary fibrosis secondary to chronic pancreatitis) and dysregulation of immune responses (such as during PBC or PSC). These effects can cause impaired hepatobiliary production and excretion of bile as well as the accumulation of bile that leads to liver tissue damage, reflux of bile constituents into the circulation, inflammation and, ultimately, biliary fibrosis[121]. The transcription of proteins such as BSEP and CYP7A1, which are involved in hepatobiliary transport systems, bile acid synthesis, detoxification and fibrogenesis, is controlled via the nuclear receptors farnesoid X receptor (FXR), pregnane X receptor, vitamin D receptor (VDR) and constitutive androstane receptor (CAR)[122–124], which have a critical role in the pathogenesis of cholestatic liver fibrosis.

Several fibrogenic conditions associated with cholestatic liver injury have been described in patients. PBC is characterized by chronic small bile duct cholangitis and is considered an autoimmune disease because of its association with a specific antimitochondrial antibody to the pyruvate dehydrogenase complex E2 subunit (PDC-E2), the major PBC-specific antimitochondrial antibody autoantigen[125]. PSC is associated with chronic cholestasis and inflammation of the biliary epithelium, causing multifocal bile duct strictures that affect the entire biliary tree and liver parenchyma, leading to the development of liver fibrosis[126]. Biliary atresia is a developmental condition in newborns that results from congenital obliteration of the bile ducts, cholestasis, hepatocyte apoptosis and rapid progression to biliary fibrosis[127]. Despite intervention with hepatoportoenterostomy (also known as the Kasai procedure) owing to progressive liver fibrosis, in the USA, 50% of patients with biliary atresia require liver transplantation[128].

Regression of liver fibrosis

The crosstalk between injured hepatocytes, inflammatory cells and hepatic myofibroblasts leads to liver fibrosis. Thus, the removal of the aetiological cause underlying chronic liver injury is the primary goal of antifibrotic therapy. It was previously believed that hepatic fibrosis was not reversible[129]. However, sequential liver biopsies have documented that removing the underlying aetiological agent might reverse hepatic fibrosis in patients with secondary biliary fibrosis[130], hepatitis C[131], hepatitis B[132], NASH[133] and autoimmune hepatitis[134]. Thus, abstinence often improves hepatic fibrosis in patients with a history of chronic alcohol abuse[135]. Bariatric surgery or weight reduction was shown to effectively suppress insulin resistance, metabolic syndrome and cause regression of liver fibrosis in some patients with NASH[136]. The analysis of patient material using histology and gene expression profiling revealed that removing the underlying aetiological agent led to the suppression of pro-inflammatory or profibrogenic mediators.

In experimental models of liver fibrosis induced by CCl_4 (REFS[6,137]), ALD[5] and BDL[138], removal of the aetiological agent results in reversal of fibrosis[2]. Regression of liver fibrosis is accompanied by a decrease of pro-inflammatory cytokines (IL-6, IL-1β and TNF) and TGFβ in the liver. In the absence of TGFβ signalling, the number of activated HSCs or myofibroblasts rapidly declines and ECM production is abrogated while the production of matrix metalloproteinases that degrade collagen fibres is upregulated over that of their inhibitors (TIMP proteins)[139].

Fate of fibrogenic myofibroblasts

Although the mechanism of HSC activation is well understood, little is known about HSC biology during fibrosis regression when activated myofibroblasts decline. One of the mechanisms of cellular recycling and homeostasis that contributes to the spontaneous resolution of liver fibrosis is the apoptosis of activated HSCs[18,140]. The apoptosis of activated HSCs is orchestrated by a complex interaction between pro-apoptotic and pro-survival signals in activated HSCs in the recovering liver, in which ~50% of activated HSCs undergo apoptosis[5]. Given that HSCs possess remarkable plasticity (such as differentiation from quiescent HSCs to activated hepatic HSCs or myofibroblasts), theoretically, activated HSCs can also de-differentiate into

quiescent HSCs. Indeed, upon cessation of toxic liver injury, some of the activated HSCs escape apoptosis, downregulate fibrogenic genes and acquire a phenotype similar to but distinct from quiescent HSCs; this phenomenon was described as HSC inactivation[5]. Understanding the biology of activated HSCs will provide new targets for antifibrotic therapy. As the reversibility of fibrosis has been reported in the heart[141,142], lung[143] and kidney[144], the concepts and approaches developed in the liver might be applicable to study fibrosis in other organs.

Using Cre–loxP-based genetic labelling of myofibroblasts, which enables irreversible labelling and tracking of the cellular fate of activated HSCs and their progeny in *Col1a1*$^{Cre\text{-}YFP}$ mice, the cellular fate of HSCs or myofibroblasts during recovery from CCl_4-induced liver fibrosis was revealed[4,5]. Following HSC loss through apoptosis (around day 7 after CCl_4 cessation), the HSC population was fully restored to fulfil their function as liver pericytes and in vitamin A storage[4,5]. The HSC population in the recovered liver does not express activation markers but it does not appear to be homogeneous either. After 1 month of CCl_4 cessation, the HSC population is composed of genetically labelled HSCs with a history of collagen type I expression (45%) and newly generated HSCs with no history of collagen type I expression (55%), indicating that fibrosis resolution is associated with a dynamic renewal of HSCs emerging in the recovering liver from an unknown source. These new HSCs possess all the characteristics of quiescent cells and are indistinguishable from quiescent HSCs from treatment-naive mice[5].

HSC senescence and apoptosis

Around 50% of activated HSCs or myofibroblasts undergo cellular death[6] shortly after cessation of CCl_4-induced liver injury, including those that undergo apoptosis[145,146] (FIG. 2). Despite extensive studies, the fate of senescent cells has not been quantified due to the lack of robust, highly expressed markers of senescence for cell fate mapping of senescent cells[6,145,146].

Senescence in activated HSCs. Although still controversial, our current understanding is that the senescence of activated HSCs improves liver fibrosis by eliminating the major source of ECM. Cellular senescence is a form of proliferation arrest associated with the stable repression of E2F target genes and exhaustion of replicative potential in cells that are 'becoming old'[147,148]. Senescent activated HSCs arise in the fibrotic liver in response to chronic injury, they remain metabolically active, exhibit gene expression profiles consistent with the cell-cycle exit, they secrete a variety of biologically active factors and cytokines that mediate immune surveillance and inflammation[145,146], and regulate tissue homeostasis, fibrosis and fibrosis resolution[145]. This change in phenotype is referred to as the senescence-associated secretory phenotype. This phenotype in senescent HSCs results from the downregulation of DNase2 and TREX1, which, at normal levels, clear cytoplasmic DNA[149]. When activated HSCs become senescent, their secretion of ECM decreases so that fibrogenesis also decreases.

The senescence of HSCs is regulated via the activation of p53-dependent and p16-dependent mechanisms. Mice deficient in *Trp53* and *Cdkn2a* (genes encoding p53 and p16, respectively) exhibit a defect in activated HSC senescence and are more susceptible to liver fibrosis[145]. The senescence of activated HSCs is associated with telomere shortening and the overexpression of telomerase prevents senescence[145,146]. In mice, IL-22 signalling through the IL-22 or IL-10 receptors on HSCs activates SOCS3 and STAT3 to induce HSC senescence[150]. In turn, the overexpression of IL-22 induces senescence and attenuates the development of liver fibrosis in mice[150].

The number of senescent activated HSCs increases in livers recovering from liver fibrosis. Upon cessation of CCl_4 injury, senescent HSCs suppress the expression of BCL2, downregulate the secretion of ECM components, upregulate the secretion of ECM-degrading enzymes and upregulate surface markers such as ribonucleic acid export 1 (RAE1) in mice and MHC class I polypeptide-related sequence A (MICA) in humans. They also upregulate TNF-related apoptosis-inducing ligand (TRAIL; also known as TNFSF10) receptors and secrete immuno-stimulatory cytokines that activate effector cells (natural killer (NK) cells and natural killer T (NKT) cells), which might make senescent HSCs susceptible to apoptosis[6,145,146,151–153].

Apoptosis of activated HSCs in the recovering liver. Several mechanisms are implicated in the apoptosis of activated HSCs: activation of death receptor-mediated pathways (FAS or TRAIL), caspase 3 and caspase 8, upregulation of pro-apoptotic proteins (for example, p53 and BAX), and activation of liver associated NK cells and NKT cells[152–154]. Experimental models have demonstrated that the depletion of activated HSCs results in liver fibrosis resolution. Activated HSCs can be depleted in mice by several mechanisms, including the genetic overexpression of herpes simplex virus thymidine kinase gene (HSV-Tk) in mice under control of the mouse GFAP promoter followed by the administration of ganciclovir[155] or pharmacological treatment with glyotoxin, sulfasalazine, IKK inhibitors or anti-TIMP antibodies[145,156,157]. All the methods that depleted activated HSCs ameliorated the development of liver fibrosis or accelerated the regression of liver fibrosis[145,156,157].

NK and NKT cells. Activation of the liver-associated population of NK cells may decrease liver fibrosis by killing activated HSCs. This process occurs via direct binding of the cells to the NKG2D receptors on HSCs, leading to subsequent degranulation of cytotoxic peptides (granzyme B) into the cytoplasm or via activation of the death receptors FAS, TNFR1 and TRAIL receptors on HSCs by their corresponding ligands, causing their apoptosis by a caspase 3-dependent and caspase 8-dependent pathway[153]. The TRAIL receptor is selectively expressed by activated HSCs but not by hepatocytes, making it attractive for targeting activated HSCs[153]. Furthermore, NK and NKT cells serve as a major source of IFNγ, which produces a strong antifibrogenic effect and directly induces HSC apoptosis in a STAT1-dependent manner[158].

Hepatic lymphocytes are highly enriched for NKT cells[159]. Hepatic NKT cells account for most CXCR6$^+$

cells in the mouse liver, where they patrol liver sinusoids and mediate intravascular immune surveillance[160]. NKT cells might have protective as well as pathogenic functions in liver diseases. In response to injury in mice, hepatic CXCR6^{+} NKT cells activate and secrete inflammatory cytokines, including INFγ and IL-4, which promote hepatic fibrogenesis[161]. The deficiency of CXCR6 in mice was associated with a selective and severe reduction of CD1d-reactive NKT cells (due to reduced survival) and CD8^{+} T cells (which possess highly antifibrotic properties), so that CXCR6$^{-/-}$ mice have reduced T cell-induced hepatitis and liver fibrosis in response to injury[161]. Furthermore, CD1d-knockout mice that lack mature CD1d-restricted NKT cells developed minimal hepatic inflammation and fibrosis in response to injury[162]. However, the selective deletion of NKT cells during regression of liver fibrosis has not been evaluated.

Macrophages. Macrophages express MMP9 and TRAIL, which promote myofibroblast apoptosis[72]. Furthermore, phagocytosis of apoptotic cells by macrophages induces MMP expression and augments ECM degradation in rodent models of fibrosis resolution[72,163]. Increased collagenase activity is a primary pathway of fibrosis resolution. At the resolution stage, activated macrophages secrete matrix metalloproteinases (for example, MMP12 and MMP13) that are responsible for matrix degradation[13,164]. Moreover, the increased activity of collagen-degrading enzymes correlates with decreased TIMP expression. TIMPs promote pro-survival signals in activated HSCs as well as activation of NF-κB, which mediates activated HSC survival via the upregulation of BCL2 and downregulation of pro-apoptotic BAX and PUMA as well as p53 expression[156,165]. Thus, pharmacological inhibition of IKK (that is a part of the upstream NF-κB signal transduction cascade) and TIMPs was effective in causing regression of liver fibrosis in mice[156,165].

Inactivation of HSCs or myofibroblasts

We and others have elucidated the fate of HSCs or myofibroblasts during recovery from toxin-induced liver fibrosis and demonstrated that ~50% of myofibroblasts escape apoptosis and persist in the liver as inactivated HSCs during fibrosis regression. The survival of inactivated HSCs was associated with the upregulation of the anti-apoptotic genes *HSPA1A* or *HSPA1B* in vivo and in culture[5] and with restoration of vitamin A storage in lipid droplets[5] (FIG. 3).

Similarities and differences between inactivated and quiescent HSCs. Inactivated HSCs can be distinguished from naive quiescent HSCs by their expression of inactivation-specific signature genes, by their absence of certain quiescent-specific genes, and in their ability to more rapidly reactivate into myofibroblasts in response to fibrogenic stimuli. Specifically, some quiescent-associated genes, such as *GFAP*, *Adipor1*, *Adpf* and *Dbp*, are not re-expressed in inactivated HSCs[166].

The expression of vitamin A is a key characteristic of quiescent HSCs and is also restored in HSCs upon their inactivation[5]. Although vitamin A stored in quiescent HSCs has been suggested to be hydrolysed to fuel HSC activation[167], quiescent HSCs that lack vitamin A storage did not alter their capacity to activate as myofibroblasts, indicating that vitamin A metabolism is dispensable for HSC activation[168] but might be critical for other HSC functions such as the modulation of immunotolerance[169].

In addition to differences in gene expression profile, inactivated HSCs more rapidly reactivate into myofibroblasts in response to fibrogenic stimuli (such as TGFβ) and more effectively contribute to liver fibrosis[5] as if they retain a biological memory of being activated. In support of this understanding, mice subjected to two rounds of CCl_4-induced liver injury (administered 6 months apart) develop more liver fibrosis than their age-matched littermates that were exposed only to the second round of CCl_4 (REF.[5]). This study suggests a testable hypothesis that inactivated HSCs maintain an epigenetic memory that promotes their more effective conversion to a profibrogenic phenotype.

PPARγ is a critical nuclear receptor that maintains the quiescent phenotype in HSCs and re-establishes the quiescent-like phenotype in inactivated HSCs in mice[37]. Mechanistic studies in cultured HSCs have characterized this role of PPARγ. The depletion of PPARγ in cultured HSCs produced an activated phenotype, whereas the overexpression of PPARγ in activated HSCs led to a regression to the quiescent phenotype[37]. In addition, the treatment of cultured activated HSCs with an adipocyte differentiation cocktail, by overexpression of SREBP1c or by culturing on a basement membrane-like ECM[170,171], resulted in the upregulation of adipogenic transcription factors, leading to the morphological and biochemical regression of activated HSCs to quiescent cells. HSC activation produced epigenetic events such as the methyl-CpG-binding protein 2 (MECP2)-mediated silencing of the gene encoding PPARγ[172] or the transcriptional activation of profibrogenic genes by specific histone modifications[173]. The importance of these in vitro observations on the role of PPARγ in the HSC phenotype has recently been demonstrated in vivo. Mice with the specific knockout of PPARγ in HSCs are more sensitive to CCl_4-induced liver fibrosis[174]. When the CCl_4 fibrogenic stimulus is removed, the liver fibrosis in these HSC-specific PPPARγ-knockout mice resolves more slowly than in wild-type mice[174].

Pharmacological treatment of liver fibrosis

Although several classes of pharmacological agents have been developed for the treatment of liver fibrosis, dependent on the aetiology, stage and progression of liver fibrosis[175–177], there are no current approved therapies. Here, we overview several strategies for antifibrotic therapy and provide a few examples of the pharmacological agents that are designed to either attenuate hepatocellular injury, suppress fibrogenic and inflammatory responses in the fibrotic liver, or facilitate fibrosis resolution (FIG. 4, TABLE 1).

Removing the cause of liver injury

Removal of the underlying aetiological cause of injury often results in the resolution of liver fibrosis. For example, the administration of antiviral therapy often results

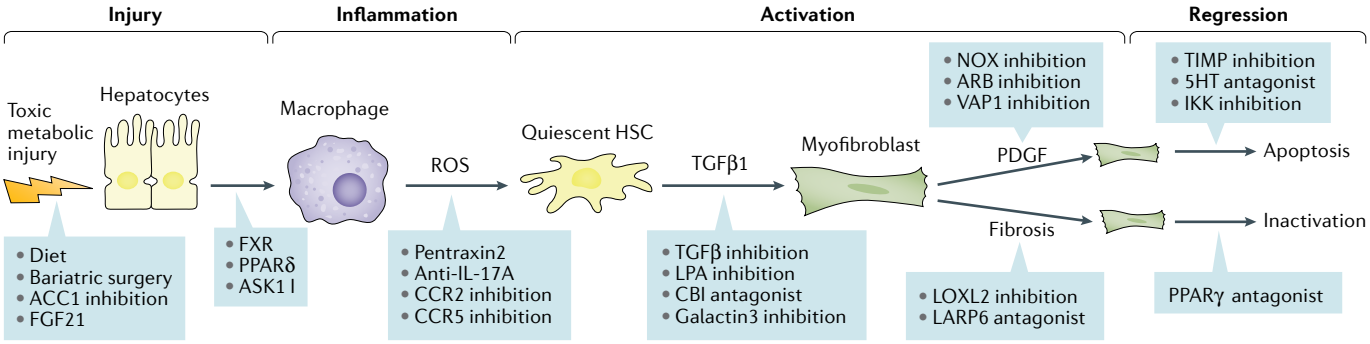

Fig. 4 | **Therapeutic opportunities for blocking fibrosis development.** Hepatocyte damage triggers inflammatory response, which leads to activation of macrophages, release of reactive oxygen species (ROS) and TGFβ1, and activation of quiescent hepatic stellate cells (HSCs) into myofibroblasts. Activated HSCs/myofibroblasts proliferate in response to various cytokines, including PDGF, secrete collagen type I and make liver fibrotic. Upon cessation of the underlying cause of injury, myofibroblasts undergo apoptosis or inactivation. Therapeutic agents that were designed to block and reverse pathological changes at each stage of liver fibrosis development and to accelerate the regression of liver fibrosis and disappearance of fibrogenic myofibroblasts are listed.

in regression of liver fibrosis in patients with HCV and HBV infection[95]. Direct-acting antiviral agents have revolutionized the treatment of HCV infection, with very high rates (>90%) of sustained virological response and a good safety profile even in the advanced stages of disease[178,179]. Direct-acting antiviral agents are the standard treatment in patients with HCV infection, including those with advanced liver disease and previous HCC[178,179]. A sustained virological response in patients with HCV infection results in protection from liver fibrosis progression[180], and some patients have decreased serum biomarkers of fibrosis such as FIB4 score and the aspartate aminotransferase to platelet ratio index[7].

Patients with NASH-associated metabolic syndrome benefit from weight loss or metabolic interventions[181]. Thus, bariatric surgery[182] or the administration of inhibitors of acetyl-CoA carboxylase that reduce liver triglycerides in individuals with fatty liver disease[183] improve liver fibrosis in patients with NASH. Fibroblast growth factor 21 (FGF21) receptor A agonists that mimic FGF21 ligand activity constitute a new class of anti-obesity and antidiabetic molecules that improve insulin sensitivity[184], ameliorate hepatic steatosis and cause weight loss, and are undergoing clinical trials in NASH and liver fibrosis.

Suppression of hepatocyte apoptosis

Apoptosis of damaged hepatocytes triggers a series of pathological events, including increased inflammation, activation of HSCs or myofibroblasts, and liver fibrosis. Dying hepatocytes upregulate intracellular expression of caspases, which mediate their cellular death, causing the release of ROS, TGFβ and other chemokines into the interstitial space and subsequent recruitment of neutrophils and monocytes to the injured liver[185]. Pharmacological inhibition of hepatocyte apoptosis by the administration of a pan-caspase inhibitor[186] or PPARδ agonist (that protects hepatocytes from cell death by reducing ROS generation by hepatocytes)[187] (TABLE 1) or FXR agonist (which regulates enterohepatic circulation and lipid homeostasis)[188] was shown to attenuate hepatic injury and fibrosis in mice. Tumour necrosis factor receptor-associated factor 1 (TRAF1) is another important regulator of immunity and inflammation, cell death, and hepatic lipid accumulation and it mediates its biological function via the activation of ASK1-mediated p38 or JNK cascades in hepatocytes[189]. ASK1 inhibitors abolish TRAF1-mediated insulin resistance of hepatocytes and suppress inflammation and liver fibrosis in experimental models of NASH[189]. Dual PPARα–PPARδ agonists[190], FXR agonists[191] and ASK1 inhibitors are undergoing clinical trials for NASH treatment[192].

Inhibiting hepatic inflammation

IL-6, TNF and TGFβ are the key pro-inflammatory and profibrogenic cytokines that drive liver fibrosis. Nevertheless, their function is critical for the maintenance of immune responses, tissue repair and homeostasis. The prolonged targeting of these cytokines is challenging due to serious adverse effects. For example, the inhibition of TNF is associated with increased risk of inflammation, whereas long-term blocking of TGFβ suppresses wound healing and promotes tumorigenesis[38]. Thus, new approaches to target inflammatory responses in the fibrotic liver are urgently needed. Successful inhibition of IL-β1 and inflammasome have been developed. The NLRP3 inflammasome, commonly known as cryopyrin[193,194], is a multiprotein complex that mediates caspase 1-dependent proteolytic maturation and secretion of IL-1β. It can be inhibited by methylsulfonylmethane[195], the inhibitor of the NALP3 inflammasome (glyburide)[196], by blocking the adaptor molecule ASC1 (which forms a complex with the NLRP3 inflammasome to activate caspase 1)[197,198] or by caspase inhibitors[199,200]. An early clinical trial with a pan-caspase inhibitor in patients with NASH demonstrated improvement in serum biomarkers of apoptosis[199,201].

The inflammasome-mediated release of IL-1β increases the transcription of its own precursor (pro-IL-1β), other inflammasome components, and inflammatory cytokines and chemokines, including TNF and CCL2 (REFS[202,203]). Thus, CC motif chemokines or their receptors (such as CCR1, CCR2 or CCR5) were identified as potential targets to suppress hepatic inflammation and fibrosis[204,205], and inhibition of these

Table 1 | **Specific inhibitors of fibrosis**

Drug types	Target effect	Clinical trial	Refs
PPARγ agonist	Block activation of HSCs	272	263,264
IL-4–IL-13 dual antibody	Multiple inflammatory mediators	NA	42
LPAR1 antagonist	Blocks HSC activation	NA	225
αv integrin inhibitors	Inhibits TGFβ activation	NA	220,221
TIMP1 antibody	Induces HSC apoptosis and blocks collagenases	NA	156
CB1 inhibitor	Indirectly blocks MF	NA	227
LOXL2 inhibitor	Blocks collagen crosslinks	273	119
NOX inhibitor	Blocks HSC activation	NA	47,230
Hedgehog inhibitor	Blocks HSC activation	NA	46,274
VAP1 inhibitor	Blocks HSC migration	NA	275
Angiotensin II receptor blocker	Blocks NOX activation	276	233,234,277
Serum amyloid P (pentraxin-2)	Blocks fibrocyte activation to myofibroblasts	NA	206,207

CB1, cannabinoid receptor 1; HSC, hepatic stellate cell; LOXL2, lysyl oxidase homologue 2; LPAR1, lysophosphatidic acid receptor 1; NA, not available; MF, myofibroblasts; NOX, NADPH oxidase; PPAR, peroxisome proliferator-activated receptor; TIMP1, metalloproteinase inhibitor 1; VAP1, Vascular adhesion protein 1.

chemokine receptors ameliorates the development of liver fibrosis. Several novel pathways have been identified to target hepatic inflammation. Serum amyloid P (SAP or pentraxin 2), a member of the pentraxin family, is produced by the liver and secreted into the blood. Circulating SAP acts as a natural inhibitor of fibrocyte activation, suppresses monocyte differentiation into fibrocytes, reduces neutrophil adhesion to ECM proteins, attenuates the activation of pro-inflammatory macrophages and promotes the phagocytosis of cellular debris[206,207]. Serum levels of SAP are reduced in patients with lung and liver fibrosis. Reconstitution of SAP ameliorates the development of bleomycin-induced lung fibrosis and liver fibrosis[206,207] (TABLE 1).

A growing body of evidence suggests that activation of IL-17 signalling promotes Kupffer cell or macrophage activation[41,208] and facilitates the development of liver fibrosis and HCC[208,209]. Several classes of T_H17 cell inhibitors (anti-IL-17A or anti-IL-23 antibodies or RORγt inhibitors or inverse agonists) have been developed and were used in patients with psoriasis and in clinical trials in patients with inflammatory bowel disease[210–212]. A recent study has demonstrated that inhibition of IL-17 signalling decreases liver fibrosis in a mouse model of combined diet-induced obesity and alcohol[213].

By contrast to IL-17, IL-22-producing T cells were shown to encourage a strong hepatoprotective effect[150]. Treatment with IL-22 ameliorates ALD due to its antioxidant, antiapoptotic, and antisteatotic effects and could become a potential therapeutic option to treat fibrogenic liver disease[214]. Finally, several other classes of molecules that potentiate the flux of inflammatory cells into the damaged liver have been identified. As an example, the vascular adhesion protein 1 (VAP1) was shown to regulate lymphocyte extravasation and recruitment to the injured liver. Antibodies to VAP1 inhibit not only lymphocyte adhesion but also the transendothelial migration of lymphocytes into the fibrotic liver, thereby suppressing hepatic inflammation in mice[215].

Inhibiting myofibroblast activation

Although TGFβ is one of the most potent stimuli of ECM synthesis, long-term therapeutic inhibition of TGFβ production is not recommended for antifibrotic therapy owing to the acceleration of proliferative and neoplastic responses in the target cells. Neutralizing anti-TGFβ antibodies or inhibition of TGFβ-activated ALK5 signalling[61] suppress the development of liver fibrosis in experimental mouse models of liver fibrosis[216,217] due to the direct inhibition of myofibroblast differentiation and proliferation. Using a combination of personalized approaches or localized drug-dosing regimens and biomarker monitoring, several TGFβ inhibitors have been tested for antifibrotic and anticancer therapy in patients[218] but have not progressed to larger scale clinical trials.

Targeting the TGFβ-associated molecules or signalling pathways provides an alternative strategy to suppress fibrosis. Pharmacological targeting of all αV integrins (and specifically αVβ6 (REF.[219])), which interacts with the latent TGFβ binding protein to activate latent TGFβ) effectively suppressed the development of liver disease and fibrogenesis in other parenchymal organs in mice[220,221]. Blocking the surface receptor CD105 on activated HSCs[222] and endothelial cells (which potentiates TGFβ signalling and TGFβ–ALK1 signalling)[223] attenuated angiogenesis, inflammation and fibrogenic responses in mice. The administration of TRC105 (a chimeric IgG1 CD105 monoclonal antibody) inhibited angiogenesis and induced the apoptosis of proliferating endothelium in patients with HCC[224].

Several other classes of molecules were successfully targeted to suppress the activation of fibrogenic myofibroblasts. Lysophosphatidic acid (LPA) is a bioactive lipid and growth factor-like mediator, which is produced mainly by activated platelets via hydrolysis of lysophosphatidylcholine by autotaxin). LPA exerts its biological activities via the stimulation of various cell types, including myofibroblasts[225]. A pan-LPA inhibitor (α-bromomethylene phosphonate-LPA) or autotaxin gene silencing was shown to inhibit inhibited liver fibrosis, activation of tumour-associated myofibroblasts and HCC[226]. Cannabinoid receptor 1 (CB1) antagonists, such as rimonabant, inhibit and reverse experimental liver fibrosis via the suppression of CB1-mediated activation of HSCs as myofibroblasts. The generation of a peripherally acting CB1 antagonist (that lacks an effect of the central nervous system) might become a treatment of choice in patients with liver fibrosis[227]. Finally, blocking VAP1 and its soluble form (which exhibits monoamine oxidase activity, has insulin-like effects and can initiate oxidative stress) using anti-VAP1 antibodies resulted in decreased liver fibrosis in mice[215]. Administration of tyrosine kinase inhibitors (sorafenib, imatinib or nilotinib, which suppress the activation of PDGF and VEGF receptors on activated HSCs)[228,229] prevents the migration, spreading, proliferation and activation of hepatic myofibroblasts and stromal cells in the injured liver.

A NOX1 or NOX4 inhibitor (GKT137831) was shown to attenuate liver fibrosis by reducing oxidative stress and ROS production in activated myofibroblasts[47,230].

The renin–angiotensin system (RAS) contributes to the pathogenesis of liver disease via the upregulation of angiotensin II signalling that stimulates the recruitment of inflammatory cells, the release of inflammatory cytokines, growth factors (such as TGFβ, IL-1β and MCP1) and ROS, and facilitates the activation of HSCs into fibrogenic myofibroblasts[231]. RAS inhibition by ACE inhibitors (ACEi; captopril, lisinopril and perindopril) and ATI receptor blockers (ARBs)[232], which prevent either the generation of angiotensin II or angiotensin peptide receptor binding and activation, have been shown to effectively inhibit the development of liver fibrosis in patients and experimental models of liver fibrosis[233,234] and suppress the production of TIMP1 in activated HSCs through PKC intracellular signalling pathways[235]. However, angiotensin-blocking agents failed to inhibit liver fibrosis in patients with HCV infection[236].

Modulation of ECM deposition

ECM proteins[237] comprise multiple, independently folded domains (composed of >300 core proteins) that provide structural support but also bind to integrin receptors, mediate cell-matrix adhesion and transduce extracellular signals to the adjacent cells[238,239]. Many growth factors (for example, FGFs or VEGFs) bind to heparin and to heparan sulfate, a component of many ECM proteoglycans. ECM domain–glycosaminoglycan chain complexes serve as a reservoir for many growth factors and are used as a platform for activation of their latent forms (such as TGFβ pro-form). For example, the binding of FGF to FGFR depends on a heparan sulfate chain[240], whereas TGFβ binding to TGFβR1 often depends on the integral membrane proteoglycans (for example, endoglin and betaglycan)[241]. Both fibronectin and vitronectin bind to HGF and form complexes of Met (the HGF receptor) and integrins (the ECM receptors), leading to enhanced cell migration[242]. Similarly, VEGF binds to specific fibronectin type III (FN3) domains in both fibronectin and tenascin-C and these associations promote cell proliferation[243]. The effects of mechanical strain were demonstrated to accelerate the activation of hepatic myofibroblasts[170,244]. The composition of the ECM also influences immune regulation. For example, intact hyaluronan interacts with regulatory T cells through CD44 and promotes their functional suppression, whereas degraded low-molecular-weight hyaluronan does not exhibit their activity[245,246]. In addition, high-molecular-weight hyaluronan promotes the differentiation of IL-10-producing cells. Hyaluronan secretion has been intimately connected with maintenance of the myofibroblast phenotype[247], and blocking hyaluronan synthesis, the inhibition of hyaluronan synthetic enzymes, HAS1 and HAS2, or the administration of an anti-CD44 antibody suppressed αSMA and TGFβ expression in myofibroblasts[248].

Collagen type I is the most abundant structural protein in the fibrotic liver. It is regulated at post-transcriptional levels (processing, transport, stabilization and translation of mRNAs) via RNA binding proteins.

Collagens become stiffer through crosslinking by lysyl oxidases (LOX), transglutaminases (TG) or non-enzymatic glycation[249,250]. Changing the quantity or the quality of collagen crosslinking might limit the regression of liver fibrosis. In the human cirrhotic liver, mature non-reducible crosslinks are higher than in non-fibrotic liver[251]. Although collagen in fibrotic septa has undergone crosslinking by TG2, TG2-deficient mice had no change in the development or regression of liver fibrosis[252]. The LOX family of enzymes are most clearly implicated in the crosslinking of fibrillary collagen type I in liver fibrosis (FIG. 4). The irreversible LOX inhibitor BAPN decreases experimental fibrosis and accelerates fibrosis regression[252,253]. The inhibition of LOXL2 (which is induced in diseased fibrogenic tissues but not present in normal tissues) using an anti-LOXL2 antibody (AB0023, simtuzumab) reduced experimental liver and lung fibrosis and decreased the TGFβ-mediated activation of myofibroblasts[119,254]. Despite its efficacy in animal models, clinical trials of simtuzumab produced no effect in patients with NASH fibrosis[255]. PAT-1251, a small molecule[256], irreversibly binds at the catalytic site of LOXL2 and blocks the oxidation of biologically relevant amines (for example, hydroxylysine, tropoelastin and fibronectin) and therefore represents a more potent inhibitor of LOXL2 activity.

The proteolytic cleavage of ECM components represents another mechanism by which the ECM is remodelled. Collagens and other ECM proteins cleaved by MMPs, ADAMs and ADAMTS proteases as well as many other proteolytic enzymes (elastases, cathepsins and various serine esterase proteases) can also act on ECM proteins[257]. TIMPs are potent MMP inhibitors[258]. In addition to their anticollagenolytic activity, TIMPs inhibit the apoptosis of activated HSCs[259]. The administration of an anti-TIMP antibody downregulates TIMPs and partially reverses experimental liver fibrosis[156].

Another class of serine proteinases, plasminogen activators, catalyse the conversion of plasminogen into plasmin, which can be processed and activated by plasma kallikrein, urokinase plasminogen activator and tissue plasminogen activator. The role of plasmin in matrix turnover is mediated both by its own proteolytic activity and by activation of matrix-degrading enzymes MMPs and TGFβ[260,261] and is controlled by a plasminogen activator inhibitor PAI1. The inhibition of PAI1 in activated HSCs suppresses the expression of ECM proteins and TGFβ, supporting a role for PAI1 in the pathogenesis of hepatic scar formation[262].

Removal of hepatic myofibroblasts

The decline of hepatic myofibroblasts during the regression of liver fibrosis can occur by two mechanisms: activated HSC apoptosis or inactivation. The inactivation of hepatic myofibroblasts into a quiescent-like state provides a new strategy for antifibrotic therapy. PPARγ ligands (such as pioglitazone, a member of the family of thiazolidinedione class of drugs) drive the inactivation of fibrogenic myofibroblasts[174]. PPARγ agonists have been studied in several trials of NASH and improve steatosis, inflammation and hepatic fibrosis[263,264]. Targeting vitamin D is another potential strategy to drive activated

HSC inactivation, as a VDR agonist (calcipotriol) was shown to limit HSC activity by suppressing TGFβ or SMAD and attenuated the development of experimental liver fibrosis[265,266]. Moreover, VDR regulates HSC activation on an epigenetic level[267]. TGFβ signalling induced the redistribution of VDR binding genome-wide sites; VDR binding to the co-regulated genes reduces SMAD3 occupancy at these sites and suppresses the activation of HSCs[268]. New studies are needed to dissect the mechanism and identify the targets that regulate the inactivation of activated HSCs or myofibroblasts.

Point of no return

Whether cirrhosis can reverse to a completely normal liver architecture remains controversial[29]. However, substantial improvement in hepatic structure and function provides evidence of regression of liver fibrosis[269]. Perhaps ECM remodelling is limited in cirrhosis by the formation of non-reducible crosslinked collagen and an ECM rich with elastin fibres that prevents its degradation. This pathophysiological state might lead to a 'point of no return' for liver fibrosis[29,269].

Because the regression of liver fibrosis depends on the collagenolytic activity of ECM-degrading MMPs, the sustained expression of TIMPs inhibits active MMP function. Moreover, a lack of ECM degradation might be caused by tissue transglutaminase or LOX, which mediates the crosslinking of ECM (preventing different types of collagens from proteolytic cleavage) and prevents HSC apoptosis[270].

Conclusions

Experimental and clinical liver fibrosis, resulting from hepatoxic or cholestatic injury, regresses with the removal of the aetiological agent. Each hepatic and recruited cell type contributes to liver fibrosis. Although there are no standard therapies to treat liver fibrosis, multiple drugs have successfully caused regression of liver fibrosis in experimental models.

Inflammation is closely associated with fibrosis. Macrophages are required not only for the initiation of liver fibrosis but also for its regression. The different macrophage populations that participate in the different steps need to be characterized, and the regulation of the switch from fibrogenic to collagenolytic needs to be elucidated. In particular, the resident macrophage Kupffer cells and the bone-marrow recruited macrophages might have entirely different functions and regulatory mechanisms.

Myofibroblasts originating from both HSCs and portal fibroblasts are reduced when fibrosis resolves. Using a variety of genetic labelling, the fate of HSCs has been determined and four phenotypes have been defined: quiescent, activated, senescent and inactivated. On the other hand, the tools to study portal fibroblasts are now being developed and their fate during liver fibrosis and its regression needs to be resolved. Similarly, the underlying mechanism of myofibroblast inactivation remains to be determined, particularly the epigenetic changes that determine the phenotype. In addition to inducing HSC apoptosis, driving the inactivation of activated HSCs or myofibroblasts might represent a novel target for the regression of fibrosis. A theoretical concern is that the resultant inactivated HSCs are more effectively reactivated than HSCs before inactivation.

Finally, liver fibrosis can also be influenced by other organs such as intestine, muscle and adipose tissues[271]. Although most of the research has been focused on HSCs or myofibroblasts and inflammatory pathways, the crosstalk between different organs, various cell types and overlapping signalling pathways should not be overlooked.

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